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Impact of Combined Aqueous Extracts of Turmeric and Curry Leaf on The Reproductive Parameters of High Salt Fed Male Wistar Rats

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Abstract
Infertility poses a significant challenge affecting millions of couples worldwide, with oxidative stress (OS) emerging as a key contributor, particularly in cases of idiopathic male infertility. This study investigated the potential therapeutic effects of combined aqueous extracts of Turmeric and Curry leaves (TCL) on reproductive parameters in high salt-fed male Wistar rats. Thirty-six rats were randomly assigned to six groups and subjected to varying interventions over a 28-day period. Sperm parameters including testosterone levels, and histological changes in testicular tissues were evaluated. Results revealed that high salt intake negatively impacted sperm quality, viability, and testosterone levels, while TCL extracts demonstrated dose-dependent improvements in these parameters. Histological analysis demonstrated varying degrees of testicular tissue distortion across groups, with TCL extracts showing protective effects against high salt-induced damage. These findings suggest that TCL extracts have potential therapeutic benefits in mitigating the adverse effects of high salt intake on male reproductive health. Further research is recommended to elucidate the underlying mechanisms and optimize the application of TCL extracts as a promising intervention for preserving fertility amidst dietary challenges.

Keywords: Curry leaves, Histological analysis, Infertility, Male reproductive health, Male Wistar rats, Turmeric

Introduction
Infertility is typically defined as the inability to achieve conception following at least one year of unprotected sexual intercourse. It is a widespread issue affecting numerous couples, often causing substantial emotional distress. The emotional toll experienced by infertile couples encompasses a range of negative feelings, including anger, depression, anguish, denial, guilt, shame, inadequacy, shock, isolation, and embarrassment (Wright *et al.,* 1991). Regrettably, a significant percentage of couples, approximately 15% to 25%, encounter difficulties in conceiving and subsequently seek medical guidance to enhance their chances of successful fertilization and pregnancy (Trussell, 2013). According to the World Health Organization (WHO) guidelines, nearly half of these cases can be attributed to male factors when there is an "alteration in sperm concentration, motility, and/or morphology in at least one sample of two sperm analyses, collected 1 to 4 weeks apart" (Agarwal and Sekhon, 2011).

The challenge intensifies when no identifiable cause of infertility can be determined. At present, oxidative stress (OS) is being explored as a significant and plausible contributor to idiopathic male infertility. OS represents an imbalance between the systemic presence of reactive oxygen species (ROS) and the biological system's capacity to effectively detoxify these reactive intermediates or repair the ensuing damage (Hampl *et al*., 2007Saalu, 2010). In a healthy physiological state, pro-oxidants and antioxidants coexist in equilibrium, and spermatozoa are equipped with antioxidant defense mechanisms to counteract ROS, safeguarding gonadal cells and mature

spermatozoa from oxidative harm (Henkel, 2011). However, under pathological conditions, the uncontrolled generation of ROS surpasses the antioxidant capabilities of seminal plasma, culminating in OS (Henkel, 2011; Trussell, 2013).

Statistical data from the United States underline OS as one of the primary causes of male infertility, with approximately 30% to 40% of infertile men exhibiting elevated ROS levels in their seminal plasma (Lanzafame *et al.,* 2009). Spermatozoa were among the first cell types identified as particularly susceptible to OS. Unfortunately, the damage incurred due to oxidants is generally irreparable in spermatozoa, mainly because they lack the necessary cytoplasmic-enzyme repair systems. This vulnerability is largely attributed to the high content of polyunsaturated fatty acids (PUFAs) in their cell membranes, rendering them extremely susceptible to oxidative damage, including lipid peroxidation (LPO). Consequently, LPO leads to the rapid depletion of intracellular adenosine tri-phosphate (ATP), causing axonemal damage, reduced sperm viability, and an increased occurrence of mid-piece sperm morphological defects, all of which contribute to diminished sperm motility (Bansal and Bilaspuri, 2011).

In contemporary times, diets have become notably characterized by their high content of salt and sugar, often referred to as the "Western diet" (Popkin, 2006). This dietary pattern aligns closely with the technological advancements that have permeated the global food system, resulting in increased food availability and accessibility. Notably, processed foods alone contribute to a substantial 80% of daily salt intake, in addition to salt used in cooking and those originating from natural sources such as meat and plant-based foods. This prevalence of salt in daily diets frequently goes unnoticed, with many individuals unaware of the substantial quantities they consume. Recent estimations suggest that the average daily salt intake per person falls within the range of 8–12 grams (Brown *et al.,* 2009), surpassing the recommended daily intake of 1.5–2.0 grams set by the World Health Organization and the Food and Agriculture Organization.

The contemporary dietary pattern characterized by processed food consumption has evolved into a significant risk factor for the development of metabolic syndrome (Chan *et al.,* 2014). Notably, high salt diets have been associated with an increasing prevalence of conditions such as hypertension and stroke (Brown *et al.,* 2009; Bibbins-Domingo et al., 2010).

One of the suggested mechanisms through which high salt diets contribute to hypertension is the generation of reactive oxygen species (ROS). When ROS production surpasses the normal capacity of cellular or exogenous antioxidants, it can lead to oxidative stress. Importantly, oxidative stress has been reported to influence the reproductive process (Sharma *et al.,* 1999). Elevated levels of ROS have been identified in 25-40% of semen samples from infertile men, often accompanied by reduced levels of antioxidants in their semen (Smith et al., 1996; Sharma *et al.,* 1999)

Throughout the course of human history, natural products have served as a primary remedy for a wide array of human health concerns (Carlson, 2010). The term "natural product" refers to any chemical substance obtained, extracted, or isolated from living organisms (Li *et al.,* 2016). The development of new drugs from these natural sources continues to present a challenging task, often commencing with the collection, extraction, isolation, purification, and characterization of the natural product. This process culminates in the determination of its pharmacological and toxicological effects. Despite the complexities involved, natural products remain a vital resource for compounds distinguished by their unique chemical structures and modes of action (Thomford *et al.,* 2018).

Turmeric, renowned as the primary source of curcumin, stands as one of the most extensively studied plants with a rich historical background in ancient Indian (Ayurveda) and Chinese medicinal applications for various therapeutic purposes (Gupta *et al.,* 2013). Curcumin, a vibrant yellow phytochemical, is derived from the rhizome of Curcuma longa from the ginger family (Zingiberaceae). Alongside curcumin, Curcuma longa also contains two other curcuminoids:

desmethoxycurcumin and bis-desmethoxycurcumin (Hewlings and Kalman, 2017).

Murraya koenigii Spreng, belonging to the Rutaceae family and commonly referred to as the curry tree, originates from Asia and is widespread across the Indian subcontinent. These plants manifest as shrubs or trees with pinnate leaves that consist of multiple leaflets, arranged alternately along the branches. The leaves, which possess glandular characteristics, offer an aromatic essence and vary in texture from leathery to membranous, while the leaflets exhibit diverse shapes with smooth or toothed edges.

The flowers, emanating in the form of a panicle, cyme, or small raceme, typically bloom at the tips of branches or in the leaf axils, some flowering individually. Possessing a fragrant quality, the flowers sport 4 or 5 sepals, along with white petals and up to 10 straight stamens. The fruit, a fleshy berry devoid of juice vesicles akin to certain related fruits, measures up to 1.3 centimetres, displaying colours ranging from orange and red to black.

In traditional practices, fresh leaves, dried leaf powder, and essential oil find widespread use in flavoring soups, curries, and various food preparations. The essential oil also serves the soap and cosmetic aromatherapy industry. Boiling curry leaves with coconut oil to a residue, used as a hair tonic, is a traditional method for maintaining natural hair colour and encouraging hair growth. Furthermore, these leaves, either whole or in parts, have been traditionally employed for their antiemetic, antidiarrheal, febrifuge, blood purifying, antifungal, depressant, anti-inflammatory, and analgesic properties, addressing concerns such as kidney pain and vomiting (Purohit *et al.,* 2009; Gandhi *et al.,* 2010).

Given the detrimental effects of high-salt diets on male reproductive health, there is a pressing need to explore interventions that could mitigate these negative impacts. Traditional antioxidants have shown promise in countering oxidative stress associated with high salt intake (Wilsons *et al.,* 2019). However, seeking alternative antioxidants, such as turmeric and curry leaf extracts, becomes crucial due to their potential efficacy and fewer side effects (Patel *et al.,* 2022). Turmeric and curry leaf extracts are rich in bioactive compounds known for their antioxidant properties (Gupta *et al.,* 2021). Investigating the impact of these natural antioxidants on male reproductive health may offer novel insights and pave the way for developing targeted interventions to alleviate the adverse effects of high-salt diets on fertility and sperm quality. Therefore, this research aimed to investigate the impact of a combined extract of turmeric and curry leaves on reproductive parameters in male Wistar rats subjected to a high-salt diet.

Materials and Methods

Research Design

In addressing our research objectives, we implemented a comprehensive preclinical animal model to investigate the impact of a combined extract of turmeric and curry leaves on reproductive parameters in male Wistar rats subjected to a high-salt diet. Drawing inspiration from validated preclinical models, we aimed to establish a robust experimental framework that aligns with the established standards in reproductive research

Procurement of Animals

Thirty-six (36) adults male Wistar rats weighing 180–200g were procured from the Animal House of the Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt. The rats served as the animal model for the study. The rats were housed in clean, disinfected wooden cages with sawdust as bedding. The animal house provided a controlled environment with a 12-hour light/dark cycle, 50–60% humidity, and a temperature of roughly 30°C. These conditions were maintained throughout the acclimatization and experimental periods. The rats were allowed to acclimatize for two weeks in the animal house. During this period, they had free access to clean water and standard animal feed. This acclimatization period ensured that the rats adapt to their new environment and stabilize before the commencement of the experiment.

Collection and Identification of Plant Materials

Fresh turmeric rhizomes and curry leaves were purchased from the local market within the University of Port Harcourt environment. The

turmeric rhizomes and curry leaves were taken to the Department of Plant Science and Biotechnology for identification and authentication before use for this study.

Preparation of Aqueous Extracts

The turmeric rhizomes were carefully cleaned to remove any dirt or debris, using clean water and a soft brush. The curry leaves were plucked from the stems and thoroughly washed to eliminate any impurities. The cleaned turmeric rhizomes and curry leaves were spread out in a single layer on clean trays or mesh screens. They were placed in a well-ventilated area with controlled temperature and airflow to facilitate drying. The turmeric rhizomes and curry leaves were left to dry naturally for 14 days (2 weeks), until they became completely dry and brittle. Once dried, the turmeric rhizomes and curry leaves were separately grounded into fine powders using an electric grinder. Maceration Method of Extraction was used.

Maceration Extraction method: the samples were weighed after the grinding and put into a maceration jar; distilled water was added to the jar in a ratio of 1:1 (the amount of is equal to the amount of the sample). The mixture was kept for 72 hours, with maceration jar tightly closed. The mixture was agitated daily. At the end of the 72 hours, the mixture was filtered and the chaff discarded. The filtrate was placed on a water bath at the temperature of 60°c and allowed to evaporate completely.

The aqueous extract was collected in airtight containers, ensuring proper labelling with relevant information such as the contents, date of preparation, and any other necessary details. The extract was stored in a cool, dry place, protected from light and moisture, to maintain its stability and bioactive properties. The entire process was carried out following standard laboratory practices and adhering to relevant safety guidelines to ensure the production of a high-quality aqueous extract of turmeric and curry leaves for further research and experimentation.

LD50 Determination of Combined Extracts of Turmeric and Curry Leaf

This was aimed to determine the lethal dose (LD50) of a combined extract solution containing turmeric and curry leaf using Locke's method for acute toxicity testing. Twelve mice were utilized in two phases of testing. In Phase 1, three groups of three mice each were administered varying doses of the combined extract solution: Group 1 (10 mg/kg), Group 2 (100 mg/kg), and Group 3 (1000 mg/kg). No fatalities were observed within 24 hours of Phase 1 testing, prompting the initiation of Phase 2. In Phase 2, three groups of one animal each were subjected to higher doses: Group 1 (1600 mg/kg), Group 2 (2900 mg/kg), and Group 3 (5000 mg/kg). Following 24 hours of observation in Phase 2, no fatalities were recorded. Consequently, the combined turmeric and curry leaf extract was deemed safe under the conditions tested.

Preparation of high salt

A solution of 1ml of 20% sodium chloride was prepared and given orally to the rats according to their weights.

Experimental Design

Thirty-six (36) adults male Wistar rats were randomly divided into six (6) groups of six (n=6) animals each. The treatment protocol is presented in the table 1 below:

Table 1. Experimental Protocol

Groups	Description	No. of Rats	Treatment Protocol
Group 1	Normal control	6	Were a dministered 2ml of normal saline and feed for 28 days
Group 2	Negative control	6	Were administered high -salt solution of 2ml of 20% sodium chloride without treatment for 28 days

Group 3	Vitamin C	6	Were administered high -salt solution of 2ml of 20% sodium chloride and 30mg/kg of vitamin C for 28 days
Group 4	Low Dose	6	Were administered high -salt solution of 2ml of 20% sodium chloride e and 250mg/kg of combined extract for 28 days
Group 5	Medium Dose	6	Were administered high -salt solution of 2ml of 20% sodium chloride and 500mg/kg of combined extract for 28 days
Group 6	High Dose	6	Were administered high -salt solution of 2ml of 20% sodium chloride and 1000mg/kg of combined extract for 28 days

The high-salt group was given 2ml of 20% NaCl solution by intragastric administration (gavage) (Dong *et al.,* 2020).

Dose Administration
The determined dose of the combined aqueous extract was administered orally to the rats in the experimental group.

Sample Collection
Sample collection involved obtaining testes, semen, and blood samples from the rats on days 14 and 28 of the experiment. These samples were collected using standard techniques and handled with appropriate precautions to ensure accurate and reliable results.

Biochemical assays
Testosterone levels:
The process of sample collection began with the extraction of blood from the rats through cardiac puncture. These blood samples were carefully deposited into designated tubes containing anticoagulants, such as EDTA, to prevent clotting. Next, the serum separation procedure commenced. The collected blood samples were left undisturbed at room temperature for approximately 30 minutes to an hour to allow clot formation. After this clotting period, the samples underwent centrifugation at high speeds, typically ranging from 1000 to 2000 x g, for 10 to 15 minutes. This centrifugation step facilitated the separation of serum from the clot. The resulting serum supernatant was meticulously transferred to clean, labelled tubes using a pipette, ensuring the avoidance of any disturbance to the clot or cellular debris.

Subsequently, the quantification of testosterone levels in the serum was conducted using the enzyme-linked immunosorbent assay (ELISA) method. ELISA relies on the specific binding of testosterone antibodies to the hormone in the sample, followed by the detection of these complexes using colorimetric or chemiluminescent substrates. Before initiating the assay, calibration curves were constructed using known concentrations of testosterone standards, spanning from low to high concentrations. These standards enabled the generation of a standard curve against which the absorbance of the sample could be compared, facilitating the determination of testosterone concentration.

For sample analysis, serum samples, along with appropriate controls and standards, were added to microplate wells. The samples underwent incubation with testosterone antibodies and detection reagents, promoting the formation of antigen-antibody complexes. Post-incubation, the wells were washed to eliminate any unbound substances, and the resulting signal, such as colour, was measured using a

spectrophotometer. Testosterone concentrations in the samples were determined by correlating their absorbance values with those of the standard curve.

Finally, data analysis was performed to calculate testosterone concentrations in the samples and express them in suitable units, such as ng/mL or nmol/L. Statistical methods, including analysis of variance (ANOVA), were employed to compare testosterone levels between experimental groups and draw conclusions regarding the effects of interventions on hormone levels.

Sperm Analysis

In the investigation of sperm morphology and vitality, the process began with the direct collection of semen from the sperm sac within the testis. This collection was carried out through precise surgical techniques, ensuring minimal disruption to the animals. Following collection, the semen samples were carefully prepared to maintain their viability and purity. Any extraneous tissue or debris was removed, and the samples were transferred to pre-warmed tubes for further analysis.

To assess sperm morphology, smears of the semen samples were prepared on glass slides and fixed using appropriate solutions. Staining techniques, such as the Diff-Quik stain, were applied to highlight sperm cell structures. These prepared slides were then examined under a light microscope to evaluate sperm morphology based on established criteria. For the assessment of sperm vitality, vital staining techniques, such as the eosin-nigrosin stain, were utilized. A portion of the semen sample was mixed with the staining solution and incubated to distinguish live sperm cells from dead ones. The stained samples were examined under a light microscope to determine the percentage of live sperm cells.

Histological Examination

The histological processing of testicular tissues from rats involved several sequential steps to prepare them for microscopic examination. Initially, the tissues were fixed to maintain their structural integrity and cellular components. Bouin's fluid served as the fixative agent, and the tissues were immersed in it for 24 hours to ensure thorough fixation. Following fixation, the tissues underwent dehydration to eliminate water and facilitate infiltration during embedding. This dehydration process involved immersion in a series of alcohol solutions with increasing concentrations. Once dehydrated, the tissues were embedded in paraffin wax, a common embedding medium in histology. The infiltrated tissues were placed in embedding molds and allowed to solidify. After embedding, the tissues were sectioned into thin slices using a microtome, ensuring precision in cutting. These tissue sections, typically 5-10 micrometres thick, were then mounted onto glass slides for further processing. The mounted tissue sections underwent staining using the Hematoxylin and Eosin (H&E) staining technique, a widely used method in histology. Hematoxylin stained the nuclei blue-purple, while Eosin stained the cytoplasm and extracellular matrix pink, providing contrast for visualization. The stained tissue sections were examined under a light microscope to assess cellular morphology, tissue architecture, and any pathological changes. Observations were recorded, and images were captured for documentation and analysis. Additionally, a specific procedure for Haematoxylin and Eosin staining was followed:

1. **Haematoxylin Staining**:
   Deparaffinization and rehydration steps were performed to remove paraffin wax and hydrate the tissues. The slides were immersed in a haematoxylin solution to stain the nuclei blue-purple. Differentiation and bluing steps were carried out to enhance contrast and coloration. Dehydration was performed to remove excess water.

2. **Eosin Staining**:
   The slides underwent staining with eosin to colour the cytoplasm and extracellular matrix pink. Dehydration and clearing steps were performed to prepare the slides for mounting.

Finally, the stained slides were mounted with a coverslip using a mounting medium to preserve the tissue sections for microscopic examination.

Ethical Considerations
The experimental protocol adhered to the ethical guidelines and regulations set by the Animal Ethics Committee of the University of Port Harcourt. The welfare and well-being of the animals were prioritized, and all procedures were conducted in accordance with animal welfare.

Method of Data Analysis
The data obtained from the biochemical assays were subjected to appropriate statistical analyses using the Statistical Package for Social Sciences (SPSS). ANOVA statistical test was employed to determine significant differences between the experimental groups. The level of significance was set at $p < 0.05$.

Results and Discussion

Results on the Effect on Sperm Parameters

Effect on Sperm Parameters:
The present study investigated the impact of Vitamin C and a combined extract of Turmeric and Curry leaves (TCL) on sperm parameters in high salt-fed Wistar rats (Tables 1 & 2) (Agarwal & Sekhon, 2010; Ross *et al*., 2010).

Sperm Appearance and Viscosity:
Across all treatment groups, the appearance of sperm remained milky, and viscosity was consistently normal, suggesting that the interventions did not alter these observable characteristics.

Sperm Volume:
Noteworthy changes in sperm volume were observed, particularly in the high salt-fed group (10% NaCl) compared to the normal control. However, the administration of Vitamin C and TCL extracts at varying dosages demonstrated potential efficacy in mitigating the adverse effects of high salt intake (Fig. 1) (Agarwal & Sekhon, 2010).

Sperm Viability and Cell Morphology:
The evaluation of sperm viability and normal cell percentage revealed significant variations. Vitamin C and TCL extracts, particularly at higher doses (150mg/kg and 200mg/kg), demonstrated a positive influence on viability and normal cell morphology (Fig 2) (Agarwal & Sekhon, 2010; Ross *et al.,* 2010).

Sperm Motility:
Active sperm cell percentages showed a dose-dependent improvement with Vitamin C and TCL extracts, indicating a potential protective effect against the deleterious consequences of high salt intake. These observations align with previous research suggesting the antioxidant properties of Vitamin C and the bioactive compounds present in turmeric and curry leaves (Fig 2) (Agarwal & Sekhon, 2010; Ross *et al.,* 2010).

Sperm Count:
Sperm count exhibited significant variations across groups, with the high salt-fed group displaying a notable reduction. Conversely, the administration of Vitamin C and TCL extracts demonstrated a dose-dependent recovery, suggesting a potential role in preserving sperm count under high salt conditions (Fig 3) (Agarwal & Sekhon, 2010; Ross *et al.*, 2010).

Effect on Testosterone Levels:
The assessment of testosterone levels indicated significant alterations associated with high salt intake. Notably, Vitamin C and TCL extracts, particularly at higher dosages, exhibited a trend towards restoring testosterone levels (Fig. 4 and Table 3) (Agarwal & Sekhon, 2010; Ross *et al*., 2010).

Table 1. Effect of Vitamin C and combined Extract of Turmeric and Curry leaves (TCL) on sperm parameters in High salt-fed Wistar rats on Day 14

Parameter	Control	10% NaCl	Vitamin C	100mg/kg Extract	150mg/kg Extract	200mg/kg Extract
Appearance	Milky	Milky	Milky	Milky	Milky	Milky
Viscosity	Normal	Normal	Normal	Normal	Normal	Normal
pH	8.0±0.00	8.0±0.00	8.0±0.00	8.0±0.00	8.0±0.00	8.0±0.00
Volume (ml)	0.27±0.03[b]	0.14±0.02[a]	0.24±0.02[b]	0.24±0.02[b]	0.26±0.02[b]	0.24±0.02[b]
Viability (%)	81.67±1.67[b]	72.00±2.56[a]	84.00±1.87[b]	80.00±3.54[b]	84.00±1.87[b]	82.00±2.55[b]
Normal cells (%)	88.33±1.67[b]	71.00±4.00[a]	86.00±1.87[b]	79.00±3.32[a,b]	84.00±1.87[b]	81.00±3.67[a,b]
Abnormal cells (%)	16.67±1.67[b]	29.00±4.00[a]	14.00±1.87[b]	21.00±3.32[a,b]	16.00±1.87[b]	19.00±3.67[b]
Active cells (%)	78.33±4.41[b]	66.00±4.30[a]	84.00±2.92[a,b]	72.00±5.15[a,b]	84.00±1.87[a,b]	77.00±3.00[b]
Sluggish cells (%)	8.33±1.67	10.00±0.00	7.00±1.23	8.00±1.23	7.00±1.23	10.00±0.00
Dead cells (%)	13.33±3.33[b]	24.00±4.30[a]	9.00±1.87[a,b]	20.00±5.48[a]	9.00±1.00[a,b]	13.00±3.00[b]
Sperm count	500±57.34[b]	280±46.37[a]	570±53.85[a,b]	450±57.01[a,b]	480±33.91[b]	460±50.99[a,b]

[a] value is significant when compared to normal control at $p<0.05$, [b] value is significant when compared to negative control (1ml of 10% NaCl) at $p<0.05$, n=4

Table 2. Effect of Vitamin C and combined Extract of Turmeric and Curry leaves (TCL) on sperm parameters in High salt-fed Wistar rats on Day 28

Parameter	Control	10% NaCl	Vitamin C	100mg/kg Extract	150mg/kg Extract	200mg/kg Extract
Appearance	Milky	Milky	Milky	Milky	Milky	Milky
Viscosity	Normal	Normal	Normal	Normal	Normal	Normal
Ph	8.0±0.00	8.0±0.00	8.0±0.00	8.0±0.00	8.0±0.00	8.0±0.00
Volume (ml)	0.27±0.03[b]	0.14±0.02[a]	0.25±0.02[b]	0.25±0.02[b]	0.26±0.02[b]	0.26±0.02[b]
Viability (%)	81.67±2.67[b]	68.00±2.56[a]	85.00±1.87[b]	80.00±3.54[b]	85.00±1.87[b]	86.00±2.55[a,b]
Normal cells (%)	88.33±1.67[b]	68.00±3.50[a]	87.00±3.87[b]	81.00±4.00[a,b]	87.00±3.87[b]	86.00±3.67[b]

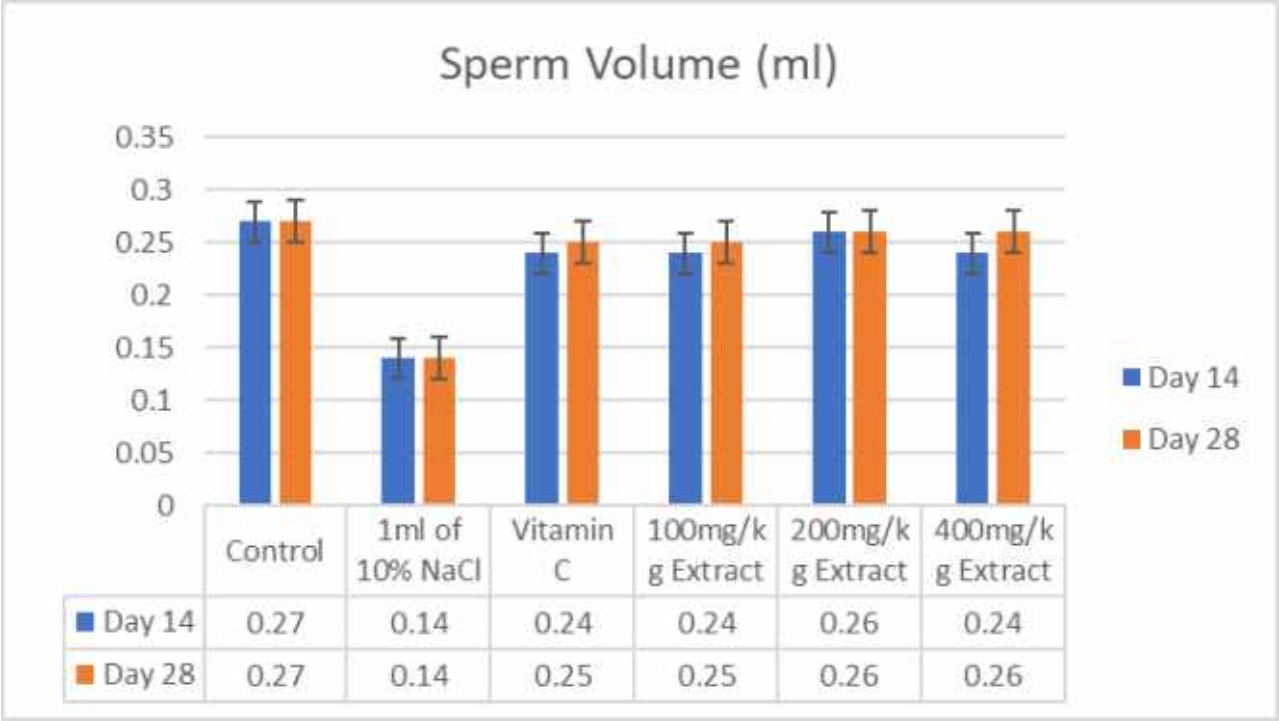
Abnormal cells (%)	16.67±1.67[b]	32.00±3.00[a]	13.00±1.87[b]	19.00±3.20[b]	14.00±1.87[b]	17.00±3.30[b]
Active cells (%)	78.33±4.41[b]	64.00±4.50[a]	86.00±4.20[a,b]	74.00±5.20[b]	86.00±.87[a,b]	79.00±5.00[b]
Sluggish cells (%)	8.33±1.67[b]	12.00±1.00[a]	7.00±1.23[b]	7.00±1.23[b]	7.00±1.23[b]	8.00±1.00[b]
Dead cells (%)	13.33±3.33[b]	26.00±4.30[a]	9.00±1.87[a,b]	15.00±5.58[b]	7.00±1.00[a,b]	10.00±3.00[b]
Sperm count	500±57.34[b]	240±42.70[a]	590±50.80[a,b]	510±55.00[b]	500±35.10[b]	495±45.00[b]

[a] value is significant when compared to normal control at $p<0.05$, [b] value is significant when compared to negative control (1ml of 10% NaCl) at $p<0.05$, n=4

Table 3. Effect of Vitamin C and combined Extract of Turmeric and Curry leaves (TCL) on Testosterone (g/dL) levels in High salt-fed Wistar rats.

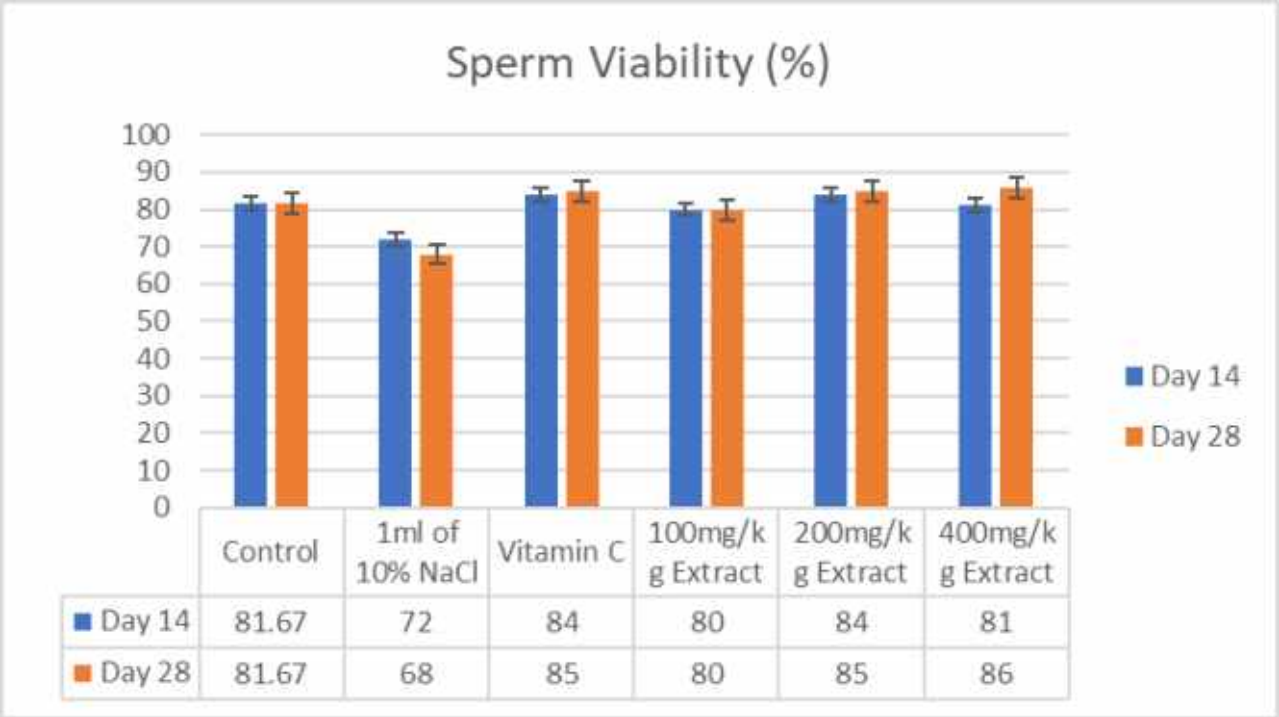
Group	Day 14	Day 28
Normal control	3.97±0.47[b]	3.97±0.47[b]
1ml of 10% NaCl	1.00±0.06[a]	1.05±0.05[a]
Vitamin C	1.26±0.05[b]	1.58±0.06[b]
100mg/kg Extract of TCL	1.97±0.42[a,b]	2.07±0.45[a,b]
200mg/kg Extract of TCL	2.32±0.27[a,b]	2.55±0.32[a,b]
400mg/kg Extract of TCL	2.35±0.44[a,b]	2.95±0.50[a,b]

[a] value is significant when compared to normal control at $p<0.05$, [b] value is significant when compared to negative control (1ml of 10% NaCl) at $p<0.05$, n=4



	Control	1ml of 10% NaCl	Vitamin C	100mg/kg Extract	200mg/kg Extract	400mg/kg Extract
Day 14	0.27	0.14	0.24	0.24	0.26	0.24
Day 28	0.27	0.14	0.25	0.25	0.26	0.26

Figure 1. Effect of Vitamin C and combined extract of Turmeric and Curry leaves on Sperm Volume (ml) in High salt-fed Wistar rats



	Control	1ml of 10% NaCl	Vitamin C	100mg/kg Extract	200mg/kg Extract	400mg/kg Extract
Day 14	81.67	72	84	80	84	81
Day 28	81.67	68	85	80	85	86

Figure 2. Effect of Vitamin C and combined extract of Turmeric and Curry leaves on Sperm Viability (%) in High salt-fed Wistar rats.

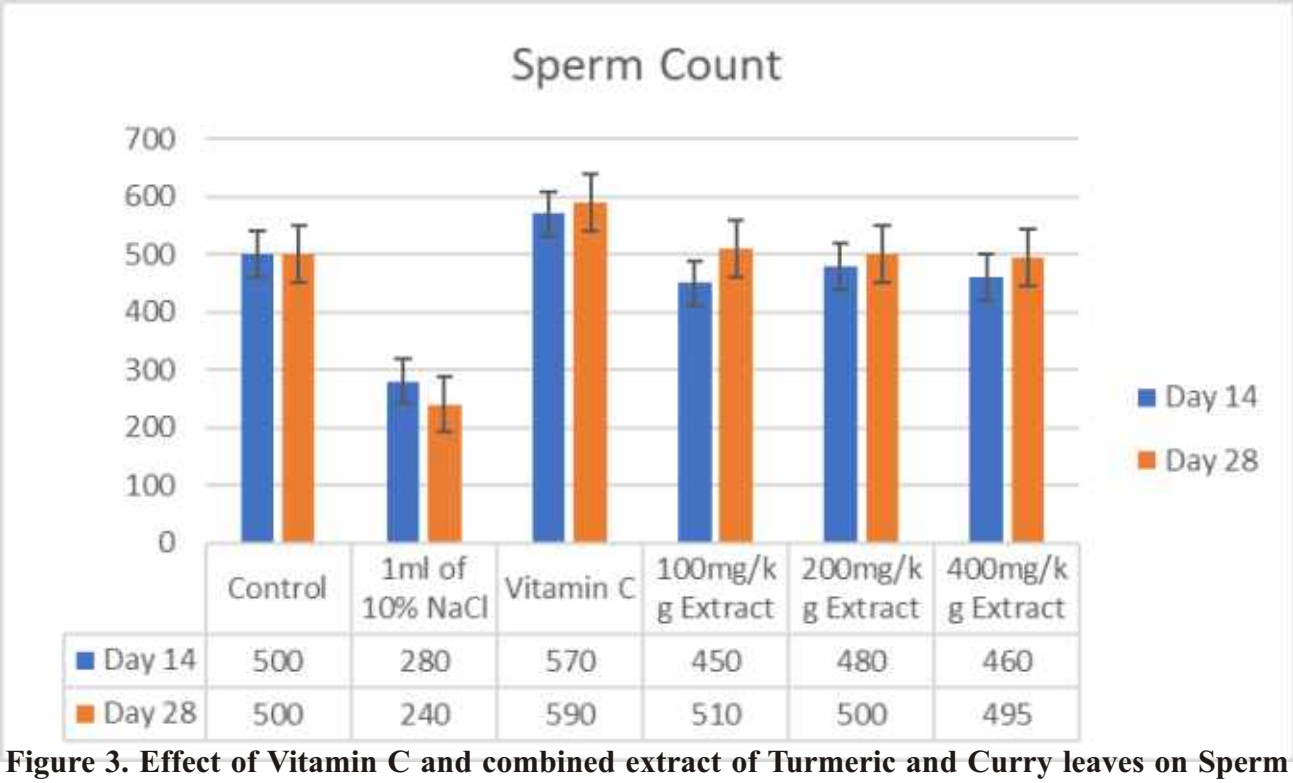


Figure 3. Effect of Vitamin C and combined extract of Turmeric and Curry leaves on Sperm Count in High salt-fed Wistar rats.

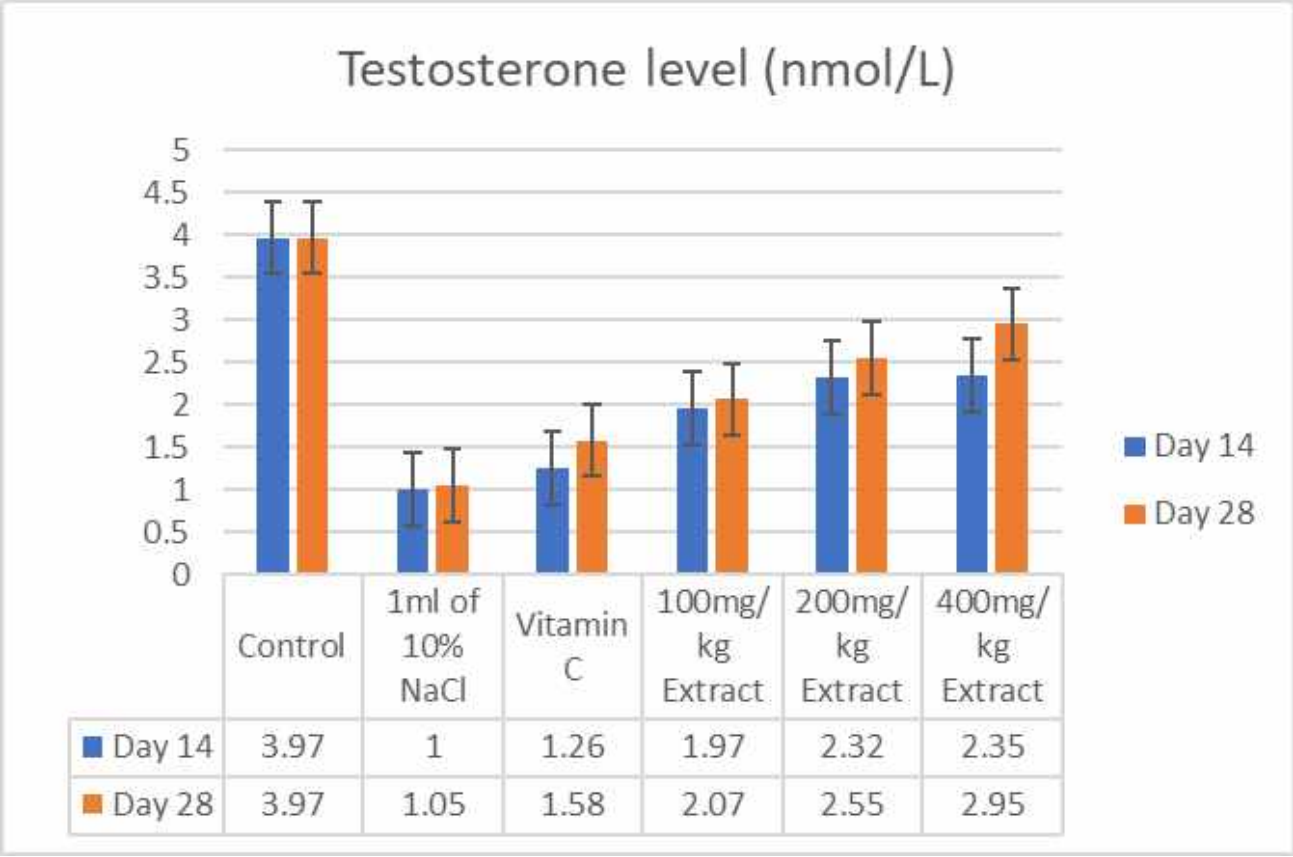


Figure 4. Effect of Vitamin C and combined extract of Turmeric and Curry leaves on Testosterone levels in High salt-fed Wistar rats.

Results on Histological Study

Results of Histological analysis after 14 Days

In Group 1 (Normal Control) (Plate 1A): The histological analysis reveals a normal appearance of testicular tissues, suggesting that the absence of experimental interventions maintains optimal spermatogenesis and testicular architecture. This showed normal appearance (Spermatogenesis) of the testis. Group 2 (1 ml of 10% NaCl): Diffused vacuolation and reduced spermatids in this group indicate hypospermatogenesis (Plate 1B) (Ali & Adel, 2013). This showed vacuolation and hypospermatogenesis of the testis. Group 3 (Vitamin C): Decreased germinal epithelium thickness, diffused vacuolation, and germinal cell loss in this group suggest that Vitamin C may not have fully protected against the detrimental effects of high salt (Plate 1C) (Agarwal & Sekhon, 2010). This indicated distortion and hypospermatogenesis of the testicular tissue.

Group 4 (100mg/kg Extract of TCL): The increase in germinal epithelium thickness with minimal distortion and visible sperm cells implies a potential protective effect of the 100mg/kg extract of TCL against the negative impacts of high salt on spermatogenesis (Plate 1D) (Ali & Adel, 2013). This indicated minimal distortion with spermatogenesis of the testicular tissue. Group 5 (200mg/kg of TCL Extract): Moderate germinal epithelia vacuolation associated with cell loss suggests a moderate distortion of testicular tissue, indicating that the 200mg/kg extract of TCL may have mitigated some adverse effects but not completely prevented them (Plate 1E) (Ali & Adel, 2013). This showed moderate distortion of the testicular tissue. Group 6 (400mg/kg TCL Extracts): The decreased germinal epithelia thickness, vacuolation, and distortion of the interstitium in this group represent a generalized distortion and hypospermatogenesis, indicating that the 400mg/kg extract of TCL might not be sufficient to prevent significant testicular damage under high salt conditions (Plate 1F) (Ali & Adel, 2013). This showed generalised distortion and hypospermatogenesis of the testicular tissue.

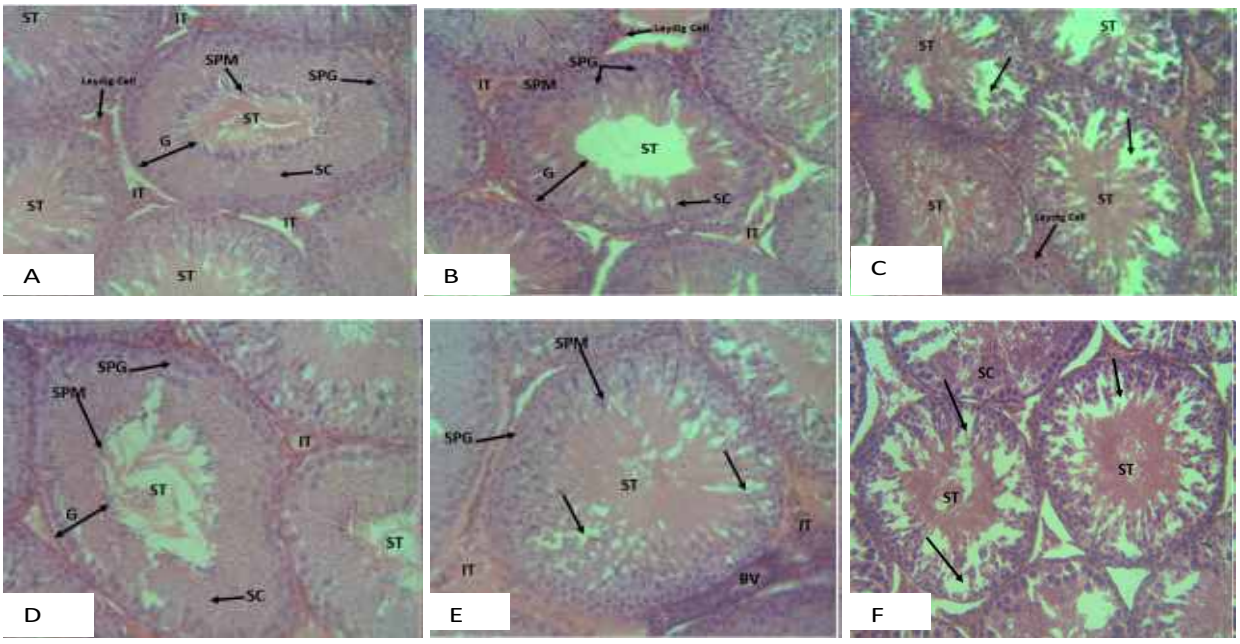


Plate 1. Photomicrograph of testis tissue showing; A - normal germinal epithelium (G) lining the seminiferous tubules (ST) with visible, sertoli cells (SC), spermatogonia (SG), spermatids (SPM) and interstitial tissue (IT) with Leydig cells (arrows); B - diffused vacuolation of the germinal epithelia lining the seminiferous tubules associated with reduced spermatids (arrows); C - the testis with decreased thickness and diffused vacuolation of the germinal epithelia associated with germinal cell loss (arrows); D - testis with increased germinal epithelia thickness with minimal distortion and visible sperm cells: testicular tissues appears normal ; E - testis with moderate germinal epithelia vacuolation associated with cell loss (arrows); F - testis with decreased germinal epithelia thickness, vacuolation associated with germinal cell loss; and distortion of the interstitium (arrows); (H&E X400)

Results of Histological Analysis after 28 Days: Group 1 (Normal Control): The sustained normal appearance of testicular tissues in the control group reinforces the absence of experimental interventions' adverse effects on spermatogenesis over time. This showed mild germinal cell loss with normal testicular appearance (Spermatogenesis). (Plate 2A). Group 2 (1 ml of 10% NaCl): Thickened germinal epithelium with mild germinal cell loss suggests a compensatory response to adverse conditions (Plate 2B). (Ali & Adel, 2013). It indicated normal appearance (Spermatogenesis) of the testis. Group 3 (Vitamin C): Decreased germinal epithelium thickness, dispersed Sertoli cells, and mild distortion of the interstitial tissue indicate a mild distortion of the testicular tissue (Plate 2C) (Agarwal & Sekhon, 2010). This showed mild Distortion of the testicular tissue. Group 4 (100mg/kg Extract of TCL): Mild germinal cell loss with decreased germinal epithelia thickness and vacuolation suggests persistent hypospermatogenesis (Plate 2D) (Ali & Adel, 2013). It showed hypospermatogenesis of the testicular tissue. Group 5 (200mg/kg of TCL Extract): Vacuolation and disruption of germinal cells resulting in germinal cell loss reflect a sustained distortion of germinal epithelia (Plate 2E) (Ali & Adel, 2013). This indicated distortion of germinal epithelia of the testicular tissue. Group 6 (400mg/kg TCL Extracts): Mild decreases in thickness and vacuolation of the germinal epithelia, along with dispersed spermatids and disrupted interstitial tissue, suggest moderate germ cell loss and testicular tissue distortion (Plate 2F) (Ali & Adel, 2013). This indicated moderate germ cell loss and testicular tissue distortion.

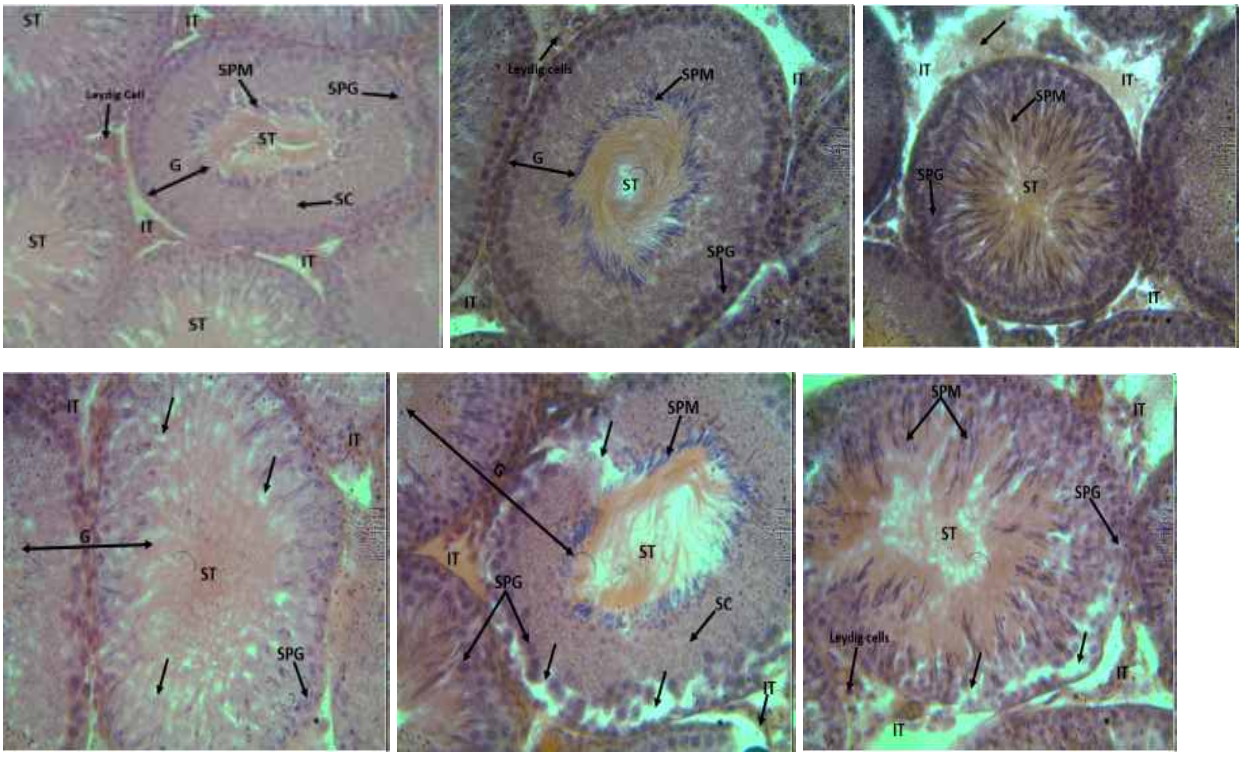


Plate 2. Photomicrograph of testis tissue showing; A - normal germinal epithelium (G) lining the seminiferous tubules (ST) with visible, Sertoli cells (SC), spermatogonia (SG), spermatids (SPM) and interstitial tissue (IT) with Leydig cells (arrows); B - thickened germinal epithelium (G) lining the seminiferous tubules (ST), Sertoli cells (SC) and spermatogonia (SPG), loss; spermatids (SPM) with interstitial tissue (IT) with Leydig cells (arrows); C - decreased thickness of the germinal epithelium (G) dispersed Sertoli cells (SC), spermatogonia (SG), spermatids (SPM) with distortion of interstitial tissue (IT) (arrows); D - testis with mild germinal cell loss associated with decreased germinal epithelia thickness and vacuolation (arrows); E - testis with vacuolation and disruption of germinal cells within the germinal epithelia resulting to germinal cell loss (arrows); F - testis with mild decreased in thickness and vacuolation of the germinal epithelia associated with dispersed spermatids; disruption of interstitial tissue (arrows).

Conclusion and Recommendation

The investigation into the impact of combined aqueous extracts of Turmeric and Curry leaves (TCL) on male reproductive parameters of high salt-fed male Wistar rats provides compelling evidence of the potential benefits of this intervention. The study revealed favourable outcomes in sperm viability, morphology, motility, and testosterone levels, particularly with the administration of TCL extracts at varying dosages. Histological assessments showcased encouraging signs of recovery and minimal distortion in testicular tissues, indicating the protective effects of TCL extracts on spermatogenesis. These findings highlight the therapeutic potential of TCL extracts in mitigating the adverse effects of high salt intake on male reproductive health. As the study contributes to our understanding of effective interventions, it opens avenues for further research to explore the underlying mechanisms and optimize the application of TCL extracts for preserving fertility in the context of dietary challenges. Encouraging further investigations to substantiate and expand upon the observed positive effects, additional research exploring the molecular mechanisms and long-term sustainability of combined aqueous extracts of Turmeric and Curry leaves (TCL) on male reproductive health in varied experimental settings is warranted.

Conflict of Interest Declaration

The authors declare that there was no conflict of interest.

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